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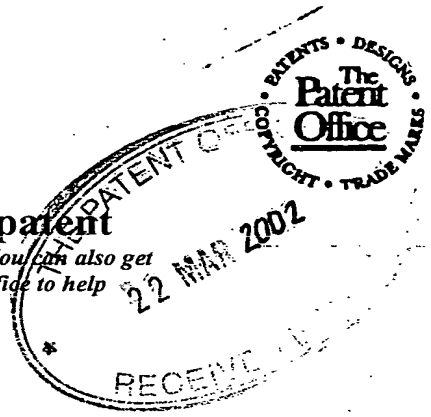

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00596007001

Medical Research Council
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If the applicant is a corporate body, give the country/state of its incorporation — Great Britain

4. Title of the invention — Chitin Microparticles and Their Medical Uses

5. Name of your agent *(if you have one)* — MEWBURN ELLIS

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Signature: Martin Ellis

Date: 21 March 2002

12. Name and daytime telephone number of person to contact in the United Kingdom: Simon Kiddle 0117 926 6411

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Chitin Microparticles and Their Medical Uses

Field of the Invention

The present invention relates to chitin microparticles and their medical uses, in particular in the treatment of allergy, conditions that would benefit from an up-regulation of the cell mediated immune system and conditions that would benefit from an up-regulation of natural killer (NK) cell activity and/or the secretion of interferon-γ (IFN-γ).

Background of the Invention

The alveolar macrophage is the most abundant leukocyte in the lumen of the alveolus and is central to the innate immune system of the lung by promoting phagocytic clearance and by the secretion of cytokines that promote an effective cell mediated immune response to inhaled particulates including microbes and pathogens. The principle cytokines produced during phagocytosis are IL-12, TNF-α, and IL-18. These macrophage cytokines subsequently induce IFN-γ production by NK cells and Th1 lymphocytes. IFN-γ acts synergistically with these cytokines to promote a Th1 cell mediated immune response and also down-regulate the production of Th2 cytokines, in particular IL-4 and IL-5 which are strong mediators of allergy.

Studies by Shibata et al (3-6), have shown that oral delivery of 1-10μm phagocytosable chitin particles results in an elevation of these Th1 cytokines in mouse spleen cell cultures. The effect was specific to the particulates as no elevation was produced by soluble chitin. It could also be reproduced in 1μm polystyrene

microspheres coated with *N*-Acetyl-D-Glucosamine, which is the main component of chitin. It was also demonstrated that oral administration of chitin down-regulates serum IgE and lung eosinophilia in a murine model of ragweed allergy (3).

Shibata et al have also developed a mouse model of allergic airway inflammation and orally administered chitin preparations to the mice (Shibata 2000). Ragweed-specific IgE levels were significantly reduced after daily oral administration of chitin to ragweed-sensitised mice, before and during immunisation. Bronchioalveolar lavage (BAL) cells were harvested 14 days after immunisation and a reduction in the levels of eosinophil and lymphocyte levels was observed after chitin treatment. Lung inflammation was determined histologically 14 days after immunisation and the peribronchial, perivascular and total lung inflammation were inhibited in the chitin-treated group.

When chitin was administered prophylactically to mice who were subsequently administered ragweed, IL-4, IL-5 and IL-10 production was significantly reduced and low but significant levels of IFN-γ were detected.

Chitin also has a prophylactic effect when administered to C57BL6 mice, which are higher responders for cell-mediated immunity/Th1 responses, but lower responders for allergic responses compared with BALB/c mice.

When ragweed-sensitised mice were treated simultaneously with ragweed and chitin, the levels of IL-4, IL-5 and IL-10 produced were significantly reduced compared to those

stimulated by ragweed alone.

However, while Shibata et al disclose the use of chitin microparticles for the treatment of allergy, the compositions are administered orally as a supplement to activate macrophages and prophylactically strengthen the immune system in the absence of recurrent bacterial infections that are decreasingly common in industrialised countries.

More generally, existing treatments for allergies typically involve the use of steroids to depress the immune system. There are undesirable side effects with steroid therapy. Synthetic drugs, such as steroids are expensive to manufacture, involving a complex process which requires complex quality control and GMP standards to meet requirements of Health and Safety Authorities. In view of these factors, it remains a problem in the art in finding effective treatments for allergy.

Summary of the Invention

Broadly, the present invention relates to the use of chitin microparticle (CMP) preparations for treating disorders by delivering the microparticles intranasally to the sinuses and upper respiratory tract, e.g. using an intranasal spray, or by inhalation, e.g. targeting alveolar macrophages in the lungs.

The macrophage has a central control function in the innate immune system of the lung by promoting phagocytic clearance of particles and by processing the presentation of inhaled allergens to lymphocytes and by secretion of cytokines that promote an effective cell mediated immune

response to inhaled particulates including microbes and allergenic substance. In particular, the present invention discloses that intranasal delivery of chitin microparticles is particularly effective in reducing a number of parameters indicative of inflammation, thus providing as alternative to steroid treatments.

The work disclosed herein arises from the finding that the intranasal delivery of chitin microparticles to mouse models of allergy produced by *Aspergillus fumigatus* (Afu) and *Dermatophagoides pteronyssinus* (Der p) is particularly effective in reducing levels of peripheral blood eosinophilia, serum total IgE, Afu-specific IgG1, the cytokine IL-4 and airway hyperresponsiveness (AHR), as well as increasing levels of the cytokines IL-12, IFN-γ and TNF-α.

Accordingly, in a first aspect, the present invention provides the use of a chitin microparticle (CMP) preparation for the preparation of a medicament for treating allergy, wherein the medicament is delivered intranasally or by inhalation.

In an alternative aspect, the present invention provides a method of treating a patient suffering from allergy, the method comprising administering to the patient a therapeutically effective amount of a chitin microparticle preparation, wherein the CMP preparation is administered intranasally or by inhalation.

Examples of allergies that can be treated according to the present invention include seasonal respiratory allergies, commonly referred to as hay fever; allergy to

aeroallergens including house mite dust, fungal spores, grass pollens, tree pollens and animal danders; allergy treatable by reducing serum IgE and eosinophilia; asthma; eczema and food allergies.

In a further aspect, the present invention provides the use of a chitin microparticle (CMP) preparation for the preparation of a medicament for the treatment of conditions that would benefit from the up-regulation of the cell-mediated immune system, wherein the medicament is administered intranasally or by inhalation.

In an alternative aspect, the present invention provides a method of treating a patient suffering from a condition that would benefit from the up-regulation of the cell-mediated immune system, the method comprising administering to the patient a therapeutically effective amount of a chitin microparticle preparation, wherein the CMP preparation is administered intranasally or by inhalation.

Examples of such conditions include the treatment of microbial infections, lung infections; pulmonary viral infections such as respiratory syncytial virus bronchiolitis, especially in infants and the elderly, or influenza virus, or rhino virus; fungal infections such as invasive pulmonary aspergillosis and invasive pulmonary candidiasis, e.g. in immunosuppressed patients; and bacterial pneumonias.

In a further aspect, the present invention provides the use of a chitin microparticle (CMP) preparation for the preparation of a medicament for the treatment of

conditions treatable by up-regulation of the activity of NK cells and/or secretion of IFN-γ by cells of the immune system, wherein the medicament is administered intranasally or by inhalation.

In an alternative aspect, the present invention provides a method of treating a patient suffering from a condition treatable by up-regulation of the activity of NK cells and/or secretion of IFN-γ by cells of the immune system, the method comprising administering to the patient a therapeutically effective amount of a chitin microparticle preparation, wherein the CMP preparation is administered intranasally or by inhalation.

An example of a condition treatable in this aspect of the invention is lung cancer.

Preferably, the medicaments set out above are for administration to humans. Preferred patient groups for intranasal treatment with CMP would include those suffering from seasonal rhinitis and sinusitis, or chronic respiratory allergies such as house dust mite allergy and who are currently taking steroids or antihistamines. Other groups include hospitalised patients being treated for chronic lung disorders including infections and lung carcinomas.

Chitin is a polymer of N-acetyl-D-glucosamine and has a similar structure to cellulose. It is an abundant polysaccharide in nature, comprising the horny substance in the exoskeletons of crab, shrimp, lobster, and insects as well as fungi. Any of these or other sources of chitin are suitable for the preparation of CMP

embodiments the device may comprise a valve between the reservoir and the delivery orifice, such that the valve can be operated to control delivery of the chitin microparticles. The microparticles may be drawn into the nose to the sinuses and upper respiratory tract or through the mouth to the alveolar macrophages by inhalation and/or by a propellant. A particularly preferred form of device is a nasal spray bottle containing a CMP preparation and optionally a carrier, the spray bottle having a neck adapted for nasal delivery.

In addition to chitin microparticles, the CMP preparations can comprise one or more of a pharmaceutically acceptable excipient, carrier, propellant, buffer, stabiliser, isotonicizing agent, preservative or anti-oxidant or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient.

Preservatives are generally included in pharmaceutical compositions to retard microbial growth, extending the shelf life of the compositions and allowing multiple use packaging. Examples of preservatives include phenol, meta-cresol, benzyl alcohol, para-hydroxybenzoic acid and its esters, methyl paraben, propyl paraben, benzalconium chloride and benzethonium chloride. Preservatives are typically employed in the range of about 0.1% to 1.0% (w/v).

Preferably, the pharmaceutically compositions are given to an individual in a "prophylactically effective amount"

or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual, e.g. providing alleviation of allergy or prophylaxis for an acceptable period. Typically, this will be to cause a therapeutically useful activity providing benefit to the individual. The actual amount of the compounds administered, and rate and time-course of administration, will depend on the nature and severity of the condition being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980. By way of example, and the compositions are preferably administered in dosages of between about 0.01 and 100mg of active compound per kg of body weight, and more preferably between about 0.5 and 10mg/kg of body weight. By way of example, this could be achieved using a nasal delivery bottle to deliver 4-8 doses of approximately 0.25ml of a 5 mg/ml solution of CMP particles.

Embodiments of the present invention will now be described by way of example and not limitation with reference to the accompanying figures.

Brief Description of the Figures and Tables

Figure 1 shows the results of treatment of Afu challenged

mice, with 17μg CMP which produced a significant decrease ($p<0.05$) in peripheral blood eosinophilia.

Figure 2 shows the results of treatment of Der p and Afu challenged mice with 4 daily doses of 25μg CMP which produced a significant decrease ($p<0.05$) in peripheral blood eosinophilia.

Figure 3 shows a reduction in serum total IgE ($p<0.0005$) of Afu challenged mice, after treatment with 17μg/day of CMP.

Figure 4 shows a reduction in serum total IgE ($p<0.0005$) of Afu challenged mice, after treatment with 5 daily doses of 17μg CMP and after re-challenge with allergen 1 week later.

Figure 5 shows the results of treatment of Der-p challenged mice, with 5 daily doses of 25μg CMP which produced a significant decrease ($p<0.005$) in total serum IgE.

Figure 6 shows a reduction in Afu specific IgG1 ($p<0.01$).

Figure 7 shows a reduction in Afu specific IgG1 ($p<0.001$) after treatment with 5 daily doses of 17μg CMP, and after re-challenge with allergen 1 week later.

Figure 8 shows a reduction in airway hyperresponsiveness ($p<0.01$) in mice re-challenged with the Afu antigen after treatment with CMP.

Figure 9 shows a reduction in AHR ($p<0.01$) in mice

challenged with Afu antigen, after 4 days of CMP treatment.

Figure 10 shows a reduction in AHR, in mice challenged with the Der p antigen after treatment with CMP, to increasing concentrations of methacholine.

Figure 11 shows a reduction in AHR 3 days after treatment with 25μg CMP preceded by allergen challenge (Der-CMP (0), $p<0.001$) and re-challenge 4 days later (Der-CMP(4), $p<0.001$).

Figure 12 shows a reduction in AHR in mice challenged with Der p, after 4 days of treatment with varying doses of CMP, in response to methacholine exposure.

Figure 13 shows lung sections differing in the degree of inflammation and obstruction after CMP treatment of Afu sensitised mice.

Table 1a indicates increases in the cytokines IL-12, IFN-γ and TNFα in spleen cells of mice challenged with Der p and Afu allergens, in response to treatment with CMP. Table 1b indicates a decrease in the geometric mean fluorescence of the cytokine IL-4 in response to treatment with CMP.

Detailed Description

Materials and Methods

Chitin microparticles delivered intranasally represent a new approach to stimulating cell mediated immunity and promoting anti-inflammatory responses in inflamed tissues. The present invention has the considerable

advantage that macrophages of the upper respiratory tract or alveolar macrophages can be directly targeted with chitin microparticles of the correct size using an intranasal spray and inhalation delivery respectively.

Two mouse models have been established to demonstrate the efficiency of the present invention.

The parameters measured in the present study are serum IgE and IgG1, peripheral blood eosinophilia, and AHR, which are all significantly elevated in the mouse models of allergy to Afu and Der p allergens and are all significantly reduced by intranasal treatment with CMP. Levels of the cytokines IL-12, IFN-γ and TNF-α which are reduced in the mouse model of allergy to Der p are all increased by intranasal treatment with CMP and levels of IL-4 are reduced. The proposed mode of action is that the CMP are bound by the mannose receptors of macrophages in the nasal mucosa and alveolae, which stimulates macrophages and dendritic cells to generate IL-12, TNF-α and IL-18, and reverses the suppression of IL-12 produced by allergen challenge, returning the levels to those observed in non-allergic mice. This leads to the generation of IFN-γ by NK cells and Th1 lymphocytes. The reduction in IL-4 is indicative of a modulation of the immune response from Th2 to Th1. In fact, all these cytokines and particularly IFN-γ, promote a shift in the populations of T lymphocytes from Th2 to Th1. This culminates in the observed reduction in serum IgE and eosinophilia, which are major components in allergy.

Chitin Microparticle Suspension Preparation (CMP)

Chitin microparticles were prepared from purified chitin

(Sigma-Aldrich, Poole, UK) by sonication of a suspension of 10mg/ml in endotoxin free PBS at maximum output for 10min with cooling on ice every 5min. The slurry was centrifuged at 4000 x g and washed 5 times with sterile PBS to remove soluble chitin. The suspension of micro-particles was examined by FACS and compared with 1μm and 20μm standardized beads (Polysciences, Inc., Warrington, Pennsylvania, USA). 98% of the particles were smaller than 20μm, 67% were between 1μm and 20μm and 33% were less than 1μm in size. Endotoxin was measured by the Limulus Amebocyte Lysate Assay (BioWhittaker, Wokingham, UK) and shown to be <1 EU/ml.

Allergen extracts

*Aspergillus fumigatus (*Afu*)* allergen extract from a 1-week culture filtrate was prepared as described by Arrunda et al and shown to be enriched in the allergen Asp f1 by SDS-PAGE followed by N-terminal sequencing. Extract was diluted into sterile endotoxin free PBS.

Standardized *Dermatophagoides pteronyssinus* (Der p) extract (Greer Labs, Lenoir, North Carolina, USA) containing 10000 Allergy Units (AU)/ml was diluted into sterile endotoxin free PBS.

Sensitisation

Female C57BL/6 mice were sensitised by 4 weekly i.p. injections of a mixture of allergen extract (68 AU Der p; 200μg Afu) with alum in 100μl of sterile PBS.

Allergen challenge and treatment with CMP

Sensitised mice were anaesthetized with isoflurane and challenged with 50 allergy units of Der p extract, or

10μg Afu allergen extract, in PBS given intranasally followed by intranasal doses of PBS or CMP or a particulate control (PC) of 1μm polystyrene beads in 50μl given 1-2 hours later. In a separate experiment it was shown that approximately 50% of FITC-labelled micro-beads given intranasally could be recovered from the lungs after 30min.

Peripheral blood eosinophils

Blood was collected from the tail vein of the mice (n=4-8 /group) for estimation of eosinophils. The total leukocyte count was determined by an automatic cell counter and the proportion of eosinophils was determined by differential counting of May-Grunwald-Giemsa stained blood smears. Results are expressed as 10^6 cells/ml.

Serum IgE and Afu-specific IgG1

Total serum IgE was measured by sandwhich ELISA (BD PharMingen, Cowley, UK) in blood serially diluted from a maximum dilution of 1:20 to give values, which were linear with respect to a standard curve of mouse IgE. Results are expressed in μg/ml. Antigen-specific IgG1 was measured by ELISA using 96-well plates coated with allergen extract. Antibody was detected with HRP-labelled anti-mouse IgG1. Results are expressed as relative absorbance units (OD450).

Intracellular cytokine staining.

After treatment, mice were humanely sacrificed by CO_2 asphyxiation and their spleens removed and homogenized in PBS. The homogenate was filtered and red blood cells lysed with ammonium chloride lysing reagent (BD Pharmingen, Cowley, UK) and fixed with 4% (v/v)

paraformaldehyde for 20min. The cells were washed with PBS supplemented with 3% heat inactivated fetal calf serum with 0.1%(w/v) sodium azide (FSB), re-suspended in 10% DMSO(v/v) in FSB and stored at -80°C.

Cells were permeabilized with Cytoperm wash buffer (CPB, BD Biosciences, Cowley, UK) for 15min at 4°C and aliquots of 10^6 cells were blocked by incubation for 30min at 4°C with CPB supplemented with 1%(v/v) rat IgG. Intracellular cytokines were stained with 1µg PE-conjugated anti-mouse cytokine monoclonal antibody (BD Biosciences, Cowley, UK) incubated for 60min at 4°C. The cells were washed with CPB followed by FSB and re-suspended in 500µl FSB.

Flow cytometry was performed with a FACScan flow cytometer (Beckton Dickinson, Mountain View, California, USA) using CellQuest software. Data were collected for 20000 cells. The average FSC of spleen cells was 100 in all cases. Stained cells (FSC>100, FL2>100) were gated and the proportion of these cells staining intensely for PE (PE>1000) was calculated. Results are expressed as the percentage intensely stained cells after subtraction of background fluorescence for unstained cells incubated with rat IgG (% PE>1000). For IL-4, the geometric mean fluorescence (GMF) was measured for stained cells and the background subtracted.

Lung histology

Immediately after treatment, the lungs of 2-4 mice from each treatment group were fixed in 10% neutral buffered formalin and sent for independent analysis. Lungs were embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E). The slides were evaluated for peribronchial inflammation and scores were assigned on a scale of 0-4, corresponding to a score of normal to

severe, respectively (7).

Whole body plethysmography

In this study, AHR was measured using unrestrained whole body plethysmography (2) with a four-chamber system (Buxco, Sharon, Connecticut, USA). Mice were first challenged with intranasal antigen and allowed to recover for 2 hours before being placed into the chambers and their breathing monitored for 10min. When acclimatized, their baseline response was measured for 5min. The mice were then subjected to 1min of aerosolised PBS, followed by progressively increasing doses of methacholine (5, 10, 20, 30, 40mg/ml PBS). Responses are recorded for 5min in every case with a short interval between to allow return to baseline. Responses were quantified using the measurement of enhanced expiratory pause (Penh).
Each group contained 4-8 mice. Penh is measured as the average percentage increase over the baseline value for mice in each group. Results are presented as the average percentage elevation in Penh after a challenge of methacholine. In order to determine if treatment produced a lasting effect, mice from the Der p experiment were re-challenged with allergen extract alone given intranasally, 4 days after completion of treatment.

Statistics

Results are the average for 4-8 mice/group and error bars are ± SEM. Significance was determined by Student's two tailed t-test. Significance was accepted for $p \leq 0.05$.

Results

Example 1

The effect of treatment with CMP on blood eosinophilia of animals challenged with Afu, is shown in Figure 1. Groups had received treatment for 4 days and measurements were made on day 5. The sample size was 4-5 mice/group. Error bars ± SEM. The results indicate that treatment with CMP resulted in a drop in the blood eosinophilia level to ca 0.3 x 10^6/ml, compared with test animals treated with PBS which exhibited blood eosinophilia levels of ca 0.7 x 10^6/ml.

Example 2

The effect of treatment with CMP on peripheral blood eosinophilia of mice challenged with Der p and Afu is shown in Figure 2. Groups were challenged daily with Afu or Der p extract, given intranasally, followed by intranasal treatment with 4 daily doses of 25µg CMP. The sample size was 4-8 mice/group. Error bars ± SEM. Peripheral blood eosinophilia was reduced by 36% in the Der p model and 58% in the Afu model ($p<0.05$).

Example 3

A comparison of the effect of treatment with CMP on serum IgE levels of mice challenged with Afu, is shown in Figure 3. Groups were treated for 5 days and measurements were made on blood collected 3 days later. The sample size was 4-5 mice/group. Error bars ± SEM. The results indicate that serum IgE levels 3 days after treatment with CMP are less that 5µg/ml IgE, compared with 24µg/ml IgE in test animals that had not received intranasal treatment.

Example 4

The effect of treatment with CMP on serum IgE levels of mice challenged with Afu is shown in Figure 4. Groups were challenged daily with Afu extract, given intranasally, followed by intranasal treatment with 5 daily doses of 17μg CMP 1 hour afterwards. Mice were re-challenged with 3 doses of allergen extract alone, the following week. The sample size was 4-8 mice/group. Error bars ± SEM. The results indicate a significant reduction in serum IgE ($p<0.0005$) which was maintained following re-challenge with allergen, one week later ($p<0.0005$).

Example 5

The effect of treatment with CMP on serum IgE levels of mice challenged with Der p, is shown in Figure 5. Groups were challenged daily with Der p extract, given intranasally, followed by intranasal treatment with CMP 1 hour afterwards. The sample size was 4-8 mice/group. Error bars ± SEM. Treatment with 5 daily doses of 25μg CMP produced a significant decrease in total serum IgE ($p<0.005$), measured 4 days after treatment.

Example 6

A comparison of the effect of treatment with CMP or PBS on serum IgG1 levels of mice challenged with Afu, is shown in Figure 6. Groups were treated for 5 days and measurements were made on blood collected 3 days later. The sample size was 4-5 mice/group. Error bars ± SEM. Test animals infected with Afu and subsequently treated with CMP show a four fold decrease in serum IgG1 levels compared with infected animals who did not receive CMP.

Example 7

The effect of treatment with CMP on Afu specific IgG1 levels of mice challenged with Afu, is shown in Figure 7. Groups were challenged daily with Afu extract, given intranasally, followed by intranasal treatment with 5 daily doses of 17μg CMP 1 hour afterwards. Mice were re-challenged with 3 doses of allergen extract alone the following week. The sample size was 4-8 mice/group. Error bars ± SEM. The results indicate a significant reduction in Afu-specific IgG1 ($p<0.001$) which was maintained on re-challenge with allergen, one week later ($p<0.01$).

Example 8

The effect of allergen re-challenge on AHR of mice challenged with Afu, is shown in Figure 8. Groups were challenged with 30mg/ml of methacholine and then treated for 3 days with 4 daily doses of 20μg CMP. The sample size was 4-8 mice/group. Error bars ± SEM. The results indicate that AHR was significantly reduced ($p<0.01$) in animals treated with CMP. These mice showed only a 110% increase in Penh over control mice when challenged with methacholine, compared to a 240% increase for PBS treated mice.

Example 9

Airway hyperresponsivness of mice challenged with Afu, in response to a 20mg/ml challenge of nebulized methacholine, is shown in Figure 9. Groups were given 4 daily doses of PBS or 25μg CMP intranasally. The sample size was 4-8 mice/group. Error bars ± SEM. A

significant reduction in AHR was observed in the CMP treated group ($p<0.01$). Treatment with the particulate control did not reduce AHR.

Example 10

A dose response of treatment groups challenged with Der p to nebulized methacholine is shown in Figure 10. Groups were given 4 daily doses of PBS or 25µg CMP intranasally and re-challenged 4 days after treatment with either 10, 20, 30 or 40mg/ml nebulized methacholine. The sample size was 4-8 mice/group. Error bars ± SEM. The results indicate a reduced AHR to all concentrations of methacholine tested.

Example 11

AHR of mice challenged with Der p in response to nebulized methacholine is shown in Figure 11. Groups were treated for 3 days with 25µg CMP intranasally, preceded by allergen challenge (Der p(0)) and re-challenged with allergen alone 4 days after completion of treatment with a total of 4 daily doses of 25µg CMP preceded by allergen challenge (Der-CMP(4)). Results are expressed as the elevation of Penh to 20mg/ml of methacholine and show a significant reductions in AHR on the fourth day of treatment (Der-CMP(0), $p<0.001$) and after re-challenge 4 days after treatment (Der-CMP(4), $p<0.001$).

Example 12

AHR of Der p (H=Der p) sensitised mice treated with CMP is shown in Figure 12. Groups were treated for 4 days with four different doses of CMP (5-40µg). On day 4 mice were challenged with Der p and treated 1-2h later with

CMP or a control treatment of the CMP supernatant, free of any CMP, from a 25μg/ml suspension (Psn). AHR was measured after exposure to 100mg/ml nebulized methacholine for 1.5min. P represents non-sensitised mice. Results show all doses of CMP were equally effective and suggest that a dose of five fold lower than that used in previous experiments can be used to prevent an allergic response in this model.

Example 13

Lung sections stained with haematoxylin and eosin, illustrating the differences in the degree of inflammation and obstruction of airways after treatment of Afu sensitised mice with CMP, are shown in Figure 13. The peribronchial inflammation of allergen challenged mice treated with PBS gave an average score of 2.5 compared with a score of 1 for CMP treated mice also challenged with allergen. This represents a 60% reduction in allergen induced inflammation. Non-sensitised mice treated with PBS gave a score of 0. Figure 13a shows normal mouse lung after treatment with PBS, 13b shows allergic lung treated with PBS and 13c shows allergic lung after intranasal treatment with 4 daily doses of 25μg CMP.

Example 14

The effect of treatment on IL-12, IFN-γ, TNF-α and IL-4 levels in Der-p and Afu challenged mice is shown in Table 1. Groups were given 4 daily doses of allergen extract followed by intranasal treatment with 25μg CMP or a non-specific particulate control of polystyrene microbeads (PC). Cytokine producing activity was assessed by measuring the proportion of highly stained cells positive

for the respective anti-cytokine antibody labelled with phycoerythrin. Results are shown ± SEM. IL-12 was significantly elevated by 77% (Der-CMP, $p<0.005$) in the Der p model and elevated by 43% (Afu-CMP) in the Afu model. The particulate control did not elevate IL-12 levels. IFN-γ was significantly elevated by 41% (Der-CMP, $p<0.05$) in the Der p model and by 22% (Afu-CMP, $p<0.005$) in the Afu model. TNF-α was significantly elevated by 44% (Der-CMP, $p<0.05$) in the Der p model and by 22% (Afu-CMP, $p<0.05$) in the Afu model. Comparison of the geometric mean fluorescence (GMF) of spleen cells stained for IL-4 showed a decrease of 34% (Der-CMP) in the Der p model and 27% (Afu-CMP) in the Afu model. No decrease was observed with the particulate control.

Table 1

a)	Percentage staining above isotype control (%PE>1000)							
Cytokine	**PBS**	**Der-PBS**	**Der-CMP**	**Der-PC**	**PBS**	**Afu-PBS**	**Afu-CMP**	**Afu-PC**
IL-12	3.3±0.1	0.9±0.1	4.2±0.1	1.0±0.1	2.0±1.0	11.5±6.0	19.0±7.0	12.0±4.0
IFN-γ	11.6±2.7	5.8±1.2	9.8±1.6	-	30.0±3.0	24.0±2.0	32.0±2.0	27.0±1.0
TNF-α	4.0±2.5	4.2±1.0	10.0±2.5	-	6.5±2.0	12.5±2.5	16.5±3.0	-

b)	Geometric mean fluorescence							
Cytokine	**PBS**	**Der-PBS**	**Der-CMP**	**Der-PC**	**PBS**	**Afu-PBS**	**Afu-CMP**	**Afu-PC**
IL-4	16.5±3.5	29.0±9.0	19.0±3.0	26.5±7.5	16.5±3.5	13.0±1.0	7.0±2.0	-

References

The references mentioned herein are all expressly incorporated by reference.

1. Arruda et al, J. Immunol. 149:3354-9, 1992.

2. Hamelmann et al, Am. J. Respir. Crit. Care Med. 156: 766-775, 1997.

3. Shibata et al, J. Immunol.,164: 1314-1321, 2000.

4. Shibata et al, J. Immunol., 161: 4283-8, 1998.

5. Shibata et al, Infection and Immunity, 65(5): 1734-1741, 1997.

6. Shibata et al, J. Immunol., 159: 2462-2467, 1997.

7. Sur et al, J. Immunol., 162: 6284-6293, 1999.

Claims

1. Use of a chitin microparticle preparation for the preparation of a medicament for the treatment of allergy, wherein the medicament is delivered intranasally or by inhalation.

2. The use of claim 1, wherein the allergy is seasonal respiratory allergy, allergy to aeroallergens including house dust mite, fungal spores, grass pollens, tree pollens and animal danders; allergy treatable by reducing serum IgE and eosinophilia; asthma; eczema or a food allergy.

3. Use of a chitin microparticle preparation for the preparation of a medicament for the treatment of a condition that would benefit from the up-regulation of the cell mediated immune system, wherein the medicament is administered intranasally or by inhalation.

4. The use of claim 3, wherein the condition comprises microbial infections, lung infections; pulmonary viral infections such as respiratory syncitial virus bronchiolitis, influenza virus, or rhino virus; fungal infections such as invasive pulmonary aspergillosis and invasive pulmonary candidiasis or bacterial pneumonias.

5. Use of a chitin microparticle preparation for the preparation of a medicament for the treatment of a condition by up-regulation of the activity of NK cells and/or secretion of IFN-γ by cells of the immune system, wherein the medicament is administered intranasally or by inhalation.

6. The use of claim 5, wherein the condition is lung cancer.

7. The use of any one of the preceding claims, wherein at least 90% of the chitin microparticles have a diameter within the range of 1-5μm.

8. The use of any one of the preceding claims, wherein the chitin microparticles are derived from the exoskeletons of crab, shrimp, lobster, and insects and fungi.

9. The use of any one of the preceding claims wherein the medicament is administered to a patient in a therapeutically effective amount of between 0.01 and 100mg of active compound per kg of body weight.

10. The use of any one of the preceding claims wherein the medicament is administered to humans.

11. The use of any one of the preceding claims wherein the chitin microparticle preparation comprises one or more of a pharmaceutically acceptable excipient, a carrier, a propellant, a buffer, a stabiliser, an isotonicizing agent, a preservative or an antioxidant.

12. A delivery device for the administration of the chitin microparticles of any one of the preceding claims, comprising:

a) a reservoir of chitin microparticles;

b) a delivery orifice adapted to locate in a patient's mouth or nose; and

c) a valve between the reservoir and the delivery

orifice such that the valve can be operated to control delivery of the chitin microparticles.

Figure 1

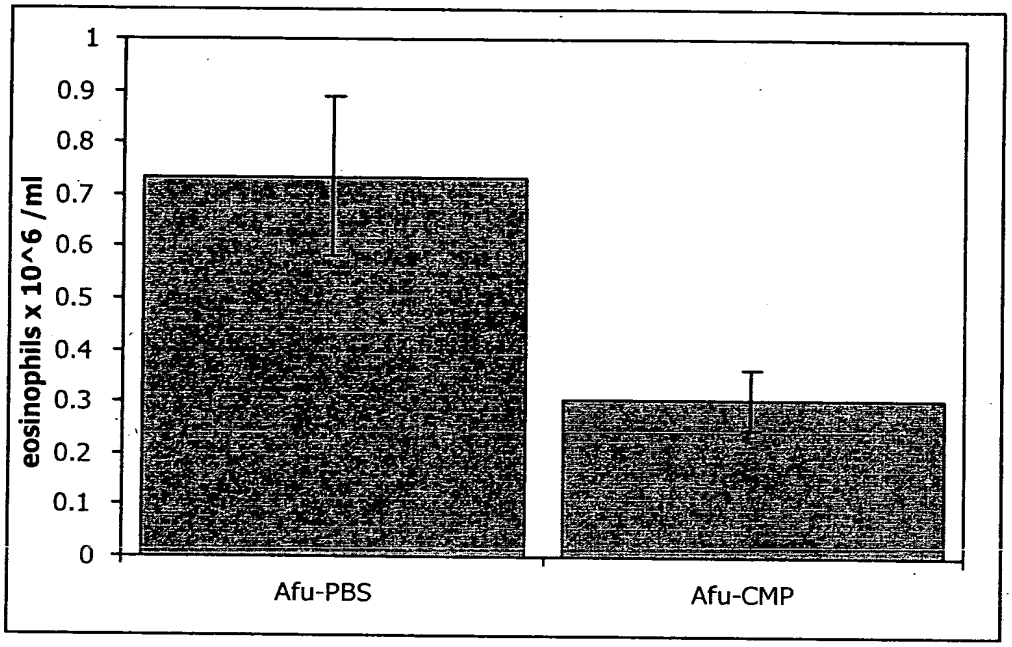


Figure 2

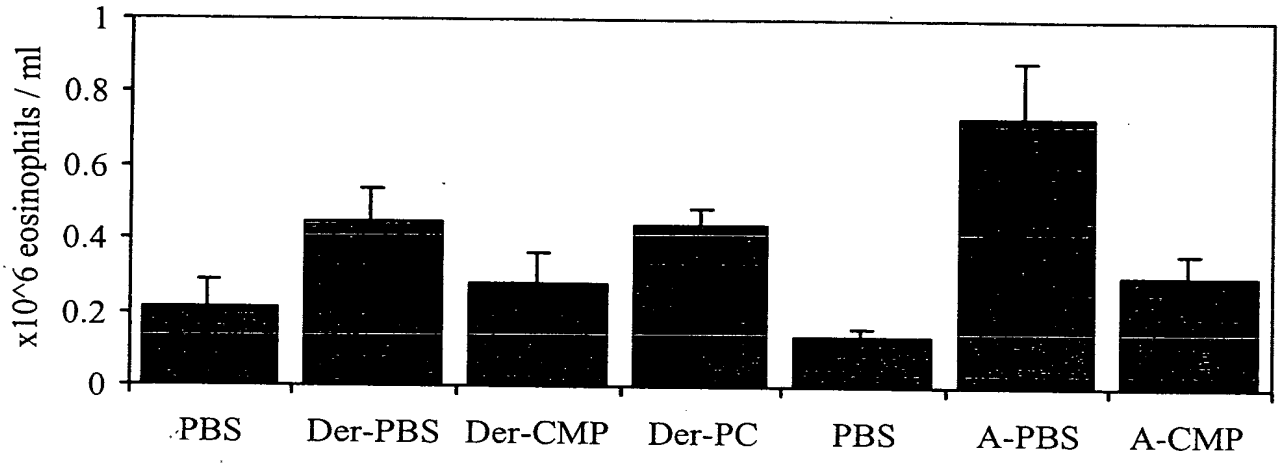
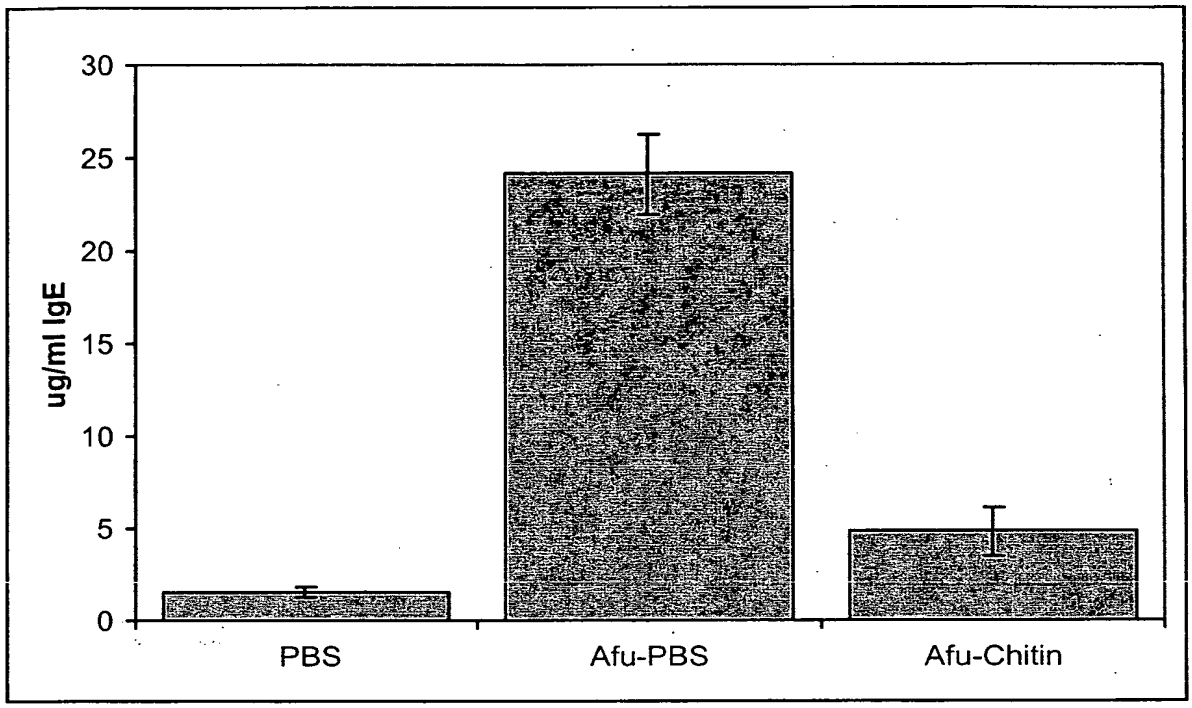


Figure 3



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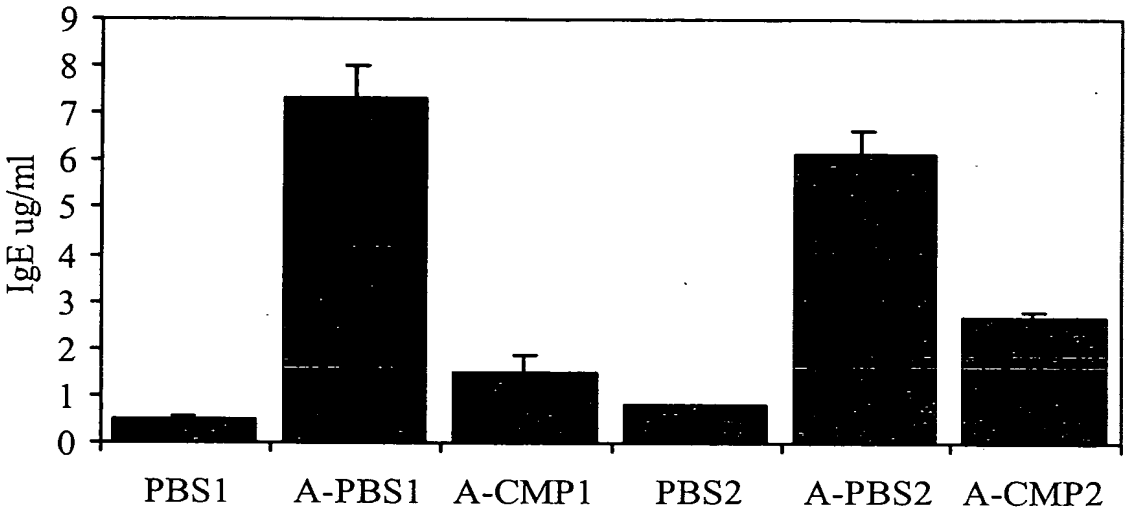

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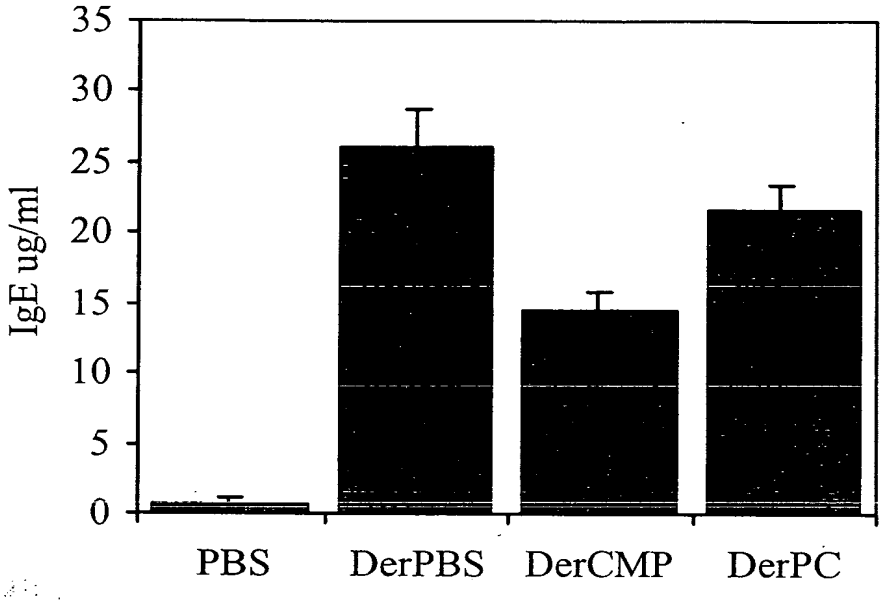
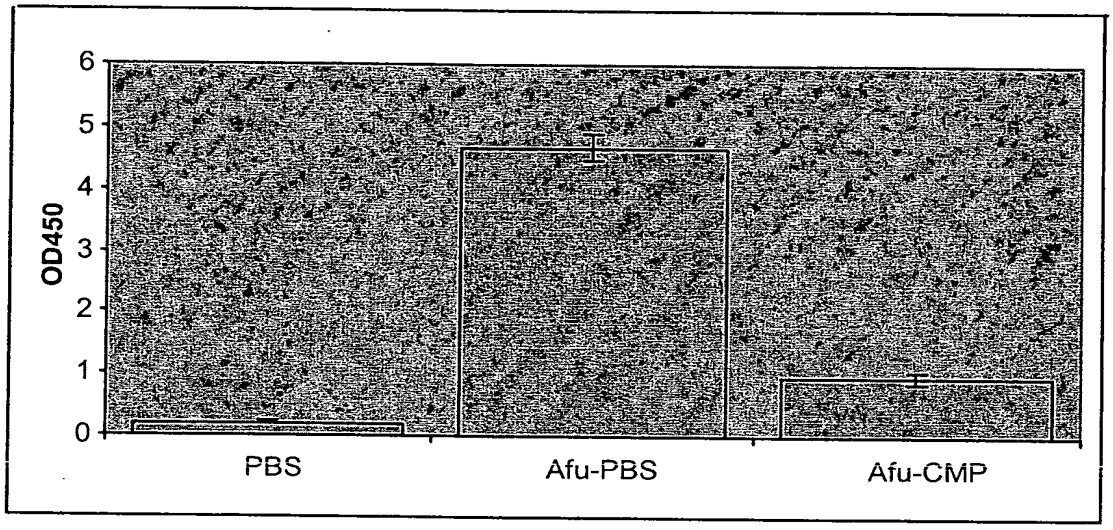


Figure 6



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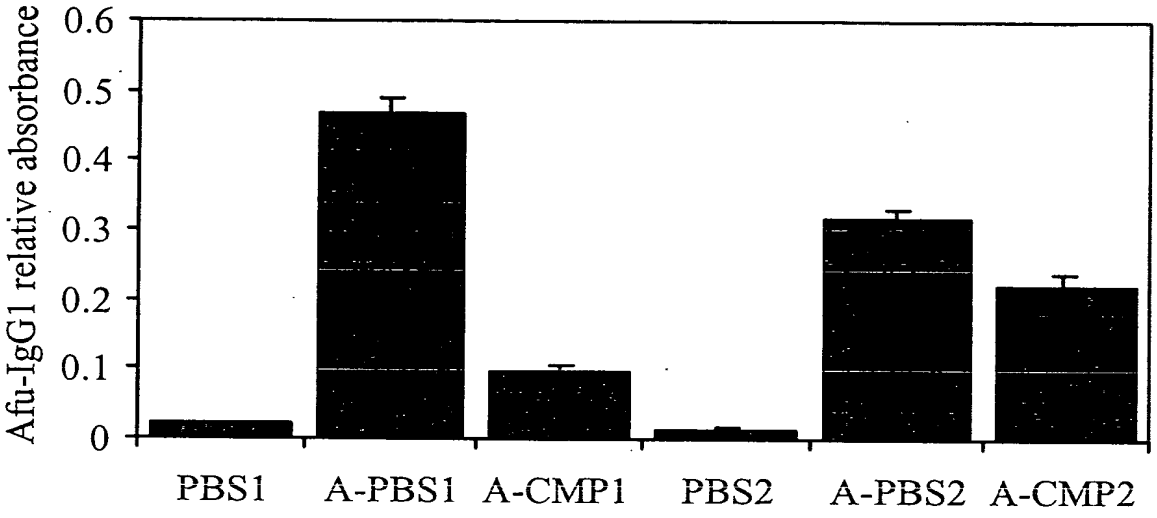

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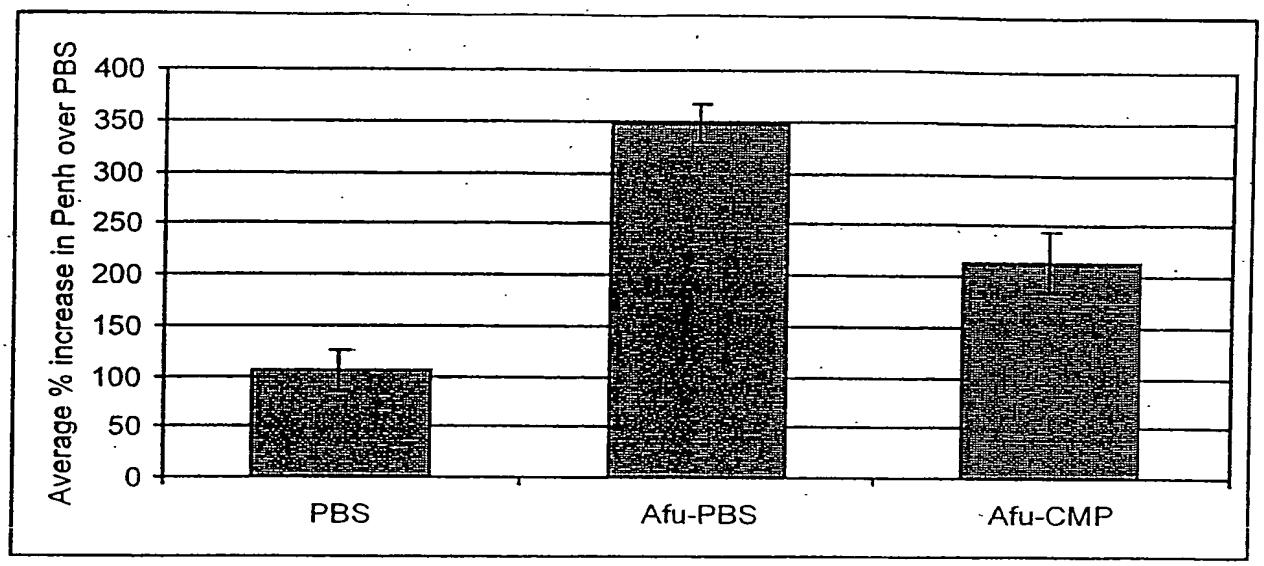

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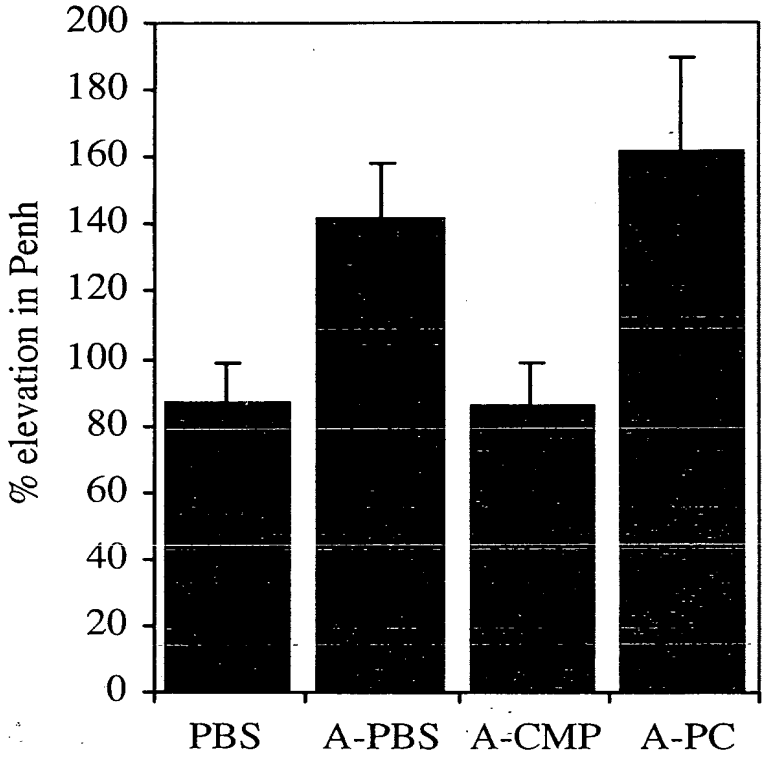
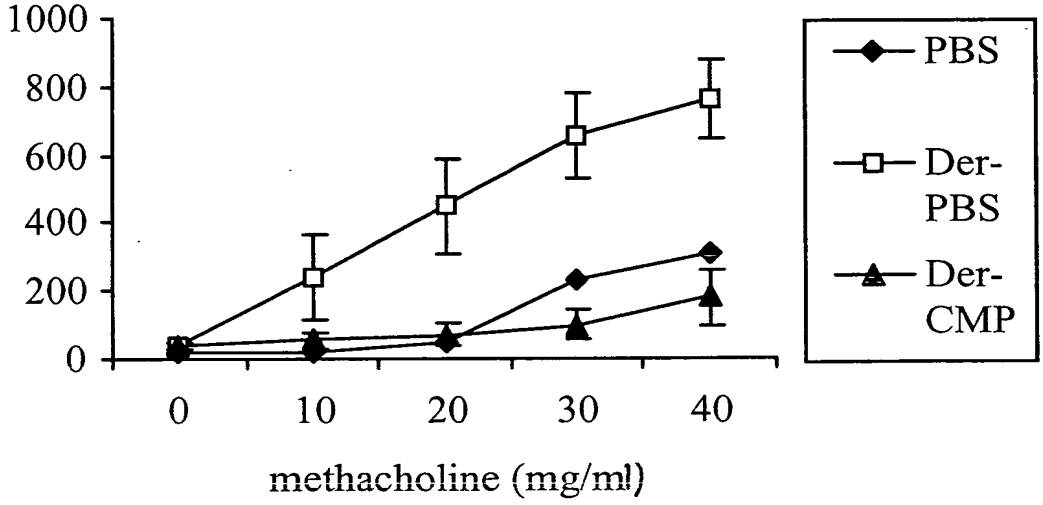


Figure 10



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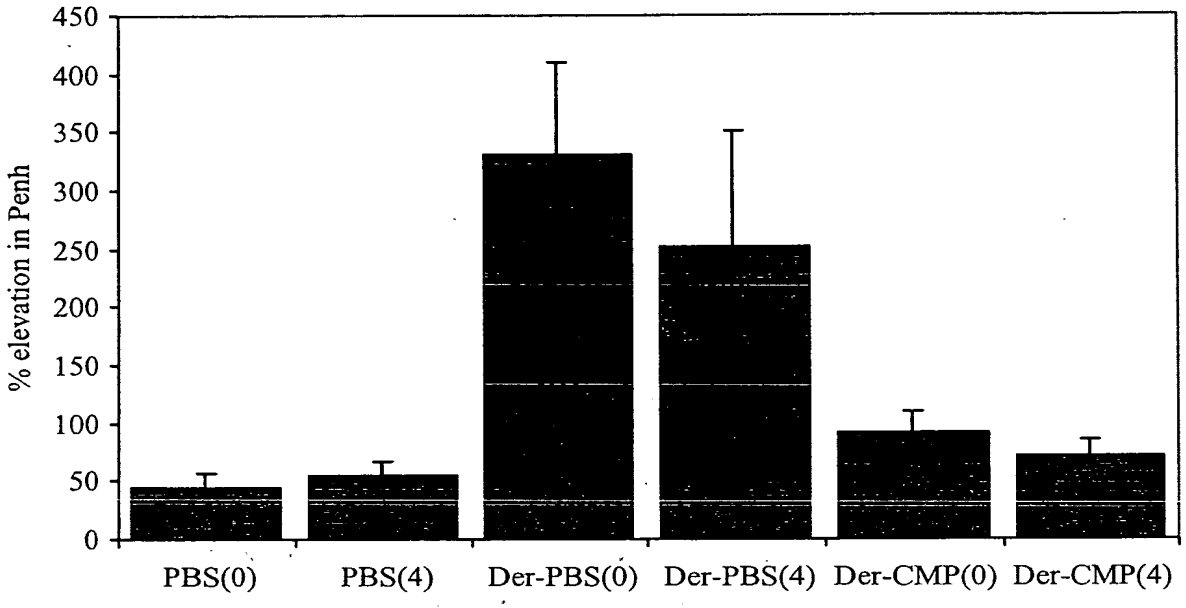

Figure 12

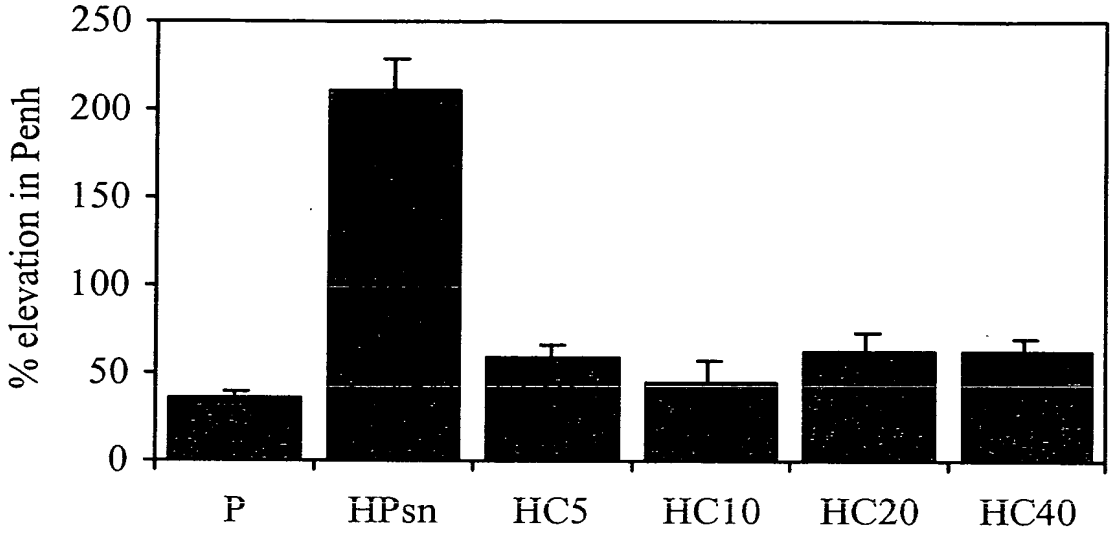

Figure 13

a

b

c